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Synthesis of bimetallic Ag-Cu nanoparticles using chemical reduction method as antibacterial agents against *Escherichia coli*

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Abstract: Bimetallic nanoparticles offer enhanced antibacterial properties due to synergistic effects between different metal ions. In this study, Ag-Cu nanoparticles were synthesized using a chemical reduction method with $NaBH_4$ as the reducing agent and PVP as a stabilizer. The resulting nanoparticles showed a greenish-black color and an average particle size of 308.8 nm with narrow distribution. Antibacterial activity against *Escherichia coli* was tested using the disc diffusion method at concentrations of 5%, 10%, and 15%. The results showed a concentration-dependent increase in inhibition zones, with a maximum average of 7.8 mm. Although lower than the positive control, the findings confirm the potential of Ag-Cu nanoparticles as alternative antibacterial agents. Further optimization is recommended for biomedical applications.

Keyword : Bimetallic nanoparticles, Ag-Cu nanoparticles, chemical reduction, antibacterial activity, Escherichia coli

1. Introduction

Bimetallic nanoparticles are nanomaterials composed of two different metallic elements. Typically, these nanoparticles exhibit a combination of the intrinsic properties of each constituent metal. Their physical and chemical characteristics can be significantly enhanced due to synergistic effects such as electron transfer, lattice strain, dual-functionality, and intermetallic interactions. The integration of two distinct metals leads to charge redistribution and changes in electronic structure, resulting in improved performance. Lattice strain, particularly in core–shell structures, can further amplify these effects. These mechanisms contribute to the enhanced catalytic activity of bimetallic nanoparticles (Duan, 2020).

Among various compositions, silver-based bimetallic nanoparticles have demonstrated remarkable catalytic and biological functionalities. These include efficient photocatalytic behavior and a broad spectrum of biological activities such as antibacterial, anticancer, antifungal, and antioxidant properties. Due to these advantages, silver-based nanoparticles are considered strong candidates for environmentally friendly technologies

and modern therapeutic applications. Various silver-based bimetallic systems have been developed for diverse applications, aiming to optimize specific functional properties that cannot be achieved by single-metal nanoparticles. Commonly studied systems include Ag-Ni, Ag-Pt, Ag-Co, and Ag-Cu (Ali, 2025).

Each combination has unique characteristics that can be tailored to meet specific needs, such as enhanced catalytic activity, chemical stability, or antibacterial efficiency. Silver-based bimetallic nanoparticles can be synthesized using a variety of methods that continue to evolve within the field of nanotechnology. These methods include chemical reduction, photochemical and chemical reactions, laser ablation, thermal decomposition, radiation-assisted synthesis, electrochemical processes, sono-chemical techniques, and microwave-assisted synthesis (Blanco-Flores, 2019).

Each synthesis approach offers specific advantages regarding efficiency, control over morphology, and stability of the final product. Selecting the appropriate synthesis method is a critical factor in determining the final characteristics of the nanoparticles, including size, shape, particle distribution, and surface structure. These attributes play an essential role in the functional performance of the nanoparticles, especially in applications such as catalysis and antibacterial activity.

Among the various metal combinations, Ag-Cu nanoparticles have received special attention due to their superior synergistic antibacterial activity and stable structural properties. Silver is widely known for its strong antimicrobial effects, while copper has high redox activity and the ability to generate reactive oxygen species (ROS), which contribute to the destruction of microbial cell walls. The combination of these two metals results in nanoparticles with significantly higher efficiency than the individual metals alone. Additionally, both Ag and Cu are relatively abundant and cost-effective, making Ag-Cu nanoparticles a promising alternative for applications in medicine and environmental technology (Saleem et al., 2024).

To obtain optimal performance, the Ag-Cu system needs to be synthesized using methods that allow precise control over size, particle distribution, and surface structure. The chemical reduction method is widely used because of its simplicity, low cost, and effectiveness in producing nanoparticles with tunable properties (Khan, 2016). Parameters such as the ratio of metal precursors, type of reducing agent, and reaction conditions can be adjusted to produce Ag-Cu bimetallic structures in the form of core-shell, alloyed, or segregated particles. Each of these structures influences antibacterial activity and particle stability in different ways.

2. Methods

The bimetallic Ag-Cu nanoparticles were synthesized using the chemical reduction method. Five grams of silver nitrate ($AgNO_3$) were used as the precursor for Ag^+ ions, and five grams of copper (II) nitrate trihydrate ($Cu(NO_3)_2 \cdot 3H_2O$) served as the precursor for Cu^{2+} ions. Each precursor was dissolved in 100 mL of isopropyl alcohol (IPA) under continuous stirring and heating using a hotplate equipped with a magnetic stirrer. Sodium borohydride ($NaBH_4$) was gradually added to the mixed solution as a reducing agent until the solution changed color to greenish-black, indicating nanoparticle formation.

Subsequently, polyvinylpyrrolidone (PVP) was introduced as a stabilizing agent, and the mixture was stirred for 2 hours at a constant temperature of 36°C.

The synthesized nanoparticles were first characterized using Particle Size Analyzer (PSA) to determine their average particle size. To evaluate antibacterial activity, the disc diffusion method was employed. The test procedure began with the preparation of Mueller-Hinton Agar (MHA) medium, which was sterilized and poured aseptically into Petri dishes. After solidification, a bacterial suspension of *Escherichia coli*, adjusted to match a 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL), was uniformly spread over the agar surface using a sterile cotton swab to ensure even bacterial distribution.

Sterile paper discs with a diameter of approximately 6 mm were immersed in Ag-Cu nanoparticle solution at a concentration of 100 µg/mL. These discs were then carefully placed on the agar surface previously inoculated with *Escherichia coli*. As controls, a negative control disc containing only the solvent without nanoparticles and a positive control disc containing a standard antibiotic such as streptomycin were used. The Petri dishes were incubated at 37°C for 18 to 24 hours. After incubation, the inhibition zones (clear zones) surrounding the discs were observed and measured. The diameter of these clear zones indicated the antibacterial effectiveness of the Ag-Cu nanoparticles in inhibiting bacterial growth around the discs.

3. Results and discussion

3.1. Synthesis results of Ag-Cu nanoparticles

The visual observation of color change during synthesis provides a preliminary indication of nanoparticle formation. Figure 1 shows the synthesized Ag-Cu nanoparticles with a distinct greenish-black color. When sodium borohydride ($NaBH_4$) was gradually added as a reducing agent, a reduction reaction occurred, converting Ag^+ ions to metallic Ag^0 and Cu^{2+} ions to metallic Cu^0. During this process, the solution color gradually changed, indicating the progression of nanoparticle formation. The emergence of a greenish-black hue is a characteristic feature of bimetallic Ag-Cu nanoparticles, as also reported by Zain (2014). This visual result is consistent with the findings of Medina et al. (2021), who obtained similarly colored Ag-Cu nanoparticles under comparable synthesis conditions. General reaction is: $Ag^+ + Cu^{2+} + NaBH_4 \rightarrow$ Ag-Cu nanoparticles + byproducts. Characterization of the synthesized nanoparticles was performed using a particle size analyzer (PSA) to evaluate their size distribution. This analysis, based on the dynamic light scattering (DLS) method, provides statistical data regarding particle size. The PSA results revealed that the Ag-Cu nanoparticles had an average particle diameter of approximately 308.8 nm. The size distribution was relatively narrow and uniform, as indicated by a single dominant peak in the spectrum. These findings suggest that the chemical reduction method was effective, producing nanoparticles with a consistent size profile. However, the measured particle size lies at the upper limit of the nanoscale, bordering on the microscale. The polydispersity index (PDI) was recorded at 4.317, indicating moderate variation in particle size.

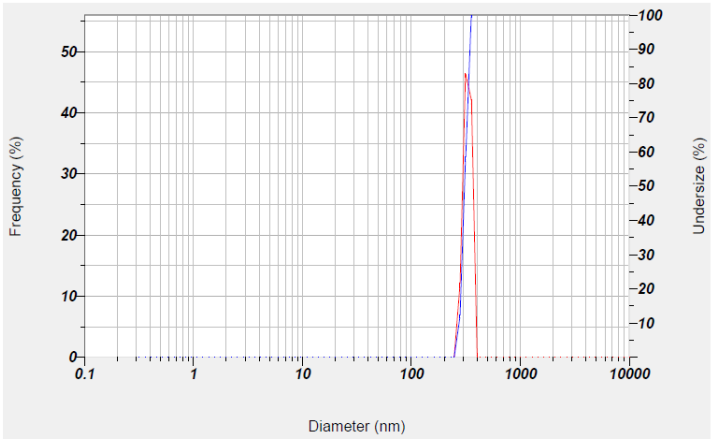

Figure 1. Particle size distribution graph of Ag-Cu nanoparticles.

Figure 1 displays the particle size distribution of the synthesized bimetallic Ag-Cu nanoparticles, with an average size of approximately 308 nm. The narrow and homogeneous distribution indicates a monodisperse system and suggests a successful synthesis process, as no secondary peaks or multimodal distributions are present. Although the particle size slightly exceeds the classical nanoscale definition (<100 nm), the characteristics remain suitable for applications such as antibacterial activity, particularly due to the potential synergistic effects between Ag and Cu.

3.2. Antibacterial activity test of Ag-Cu bimetallic nanoparticles

The antibacterial test was conducted using Ag-Cu nanoparticles at concentrations of 5%, 10%, and 15%. The evaluation used the disc diffusion method, with measurements taken three times for each concentration. Table 1 presents the inhibition zone diameters of the Ag-Cu nanoparticles against *Escherichia coli* at the various concentrations. The inhibition zone reflects the antibacterial potency of the agent. A larger zone indicates stronger antibacterial activity.

Table 1. Inhibition zone diameters against *Escherichia coli*

Ag-Cu NP Concentration	Measurement 1 (mm)	Measurement 2 (mm)	Measurement 3 (mm)	Average (mm)
5%	7.6	7.3	7.1	7.3
10%	7.8	7.5	7.3	7.5
15%	8	7.9	7.5	7.8
Positive control (K^+)	27.4	26.6	27.8	27.3
Negative control (K^-)	0	0	0	0

Based on Table 1, it is evident that increasing the concentration of Ag-Cu nanoparticles directly affects the diameter of the inhibition zones formed. At a concentration of 5%, the average inhibition zone measured 7.3 mm. When the concentration was increased to 10%, the inhibition zone expanded to an average of 7.5 mm. At the highest concentration of 15%, the zone reached an average diameter of 7.8 mm. These results indicate that higher concentrations of nanoparticles lead to larger inhibition zones, reflecting enhanced

antibacterial effectiveness against *Escherichia coli*. The antibacterial activity of Ag-Cu nanoparticles can be attributed to the ability of Ag^{+} and Cu^{2+} ions to disrupt bacterial cell wall structures, cause membrane leakage, and interfere with protein and DNA functions (Shumbula et al., 2024).

The antibacterial performance of Ag-Cu nanoparticles observed in this study reinforces their growing potential as multifunctional agents in both biomedical and environmental fields. The data indicate that inhibition zones expanded progressively with increased NP concentration, reflecting enhanced antibacterial activity. This trend is consistent with previous findings, particularly those involving bimetallic nanoparticles where synergistic interactions between Ag^{+} and Cu^{2+} ions yield improved efficacy compared to single-metal systems. As noted by Rai et al. (2021), the combined ionic effects of silver and copper amplify their disruption of bacterial physiology, from destabilizing cell membranes to interfering with key intracellular functions.

At the molecular level, Ag^{+} and Cu^{2+} ions are known to bind with thiol-containing proteins in bacterial membranes and cytoplasm. These interactions lead to the denaturation of crucial enzymes and compromise essential processes such as ATP production and DNA replication. In parallel, these nanoparticles facilitate the generation of ROS, which induce oxidative stress within microbial cells. The resulting damage to lipids, proteins, and nucleic acids can lead to rapid bacterial death. Yoon et al. (2019) emphasize that this oxidative damage is particularly effective against Gram-negative bacteria like *Escherichia coli*, where the outer membrane structure is especially vulnerable to ROS-mediated degradation.

Unlike traditional antibiotics that typically act on a single molecular target, Ag-Cu NPs employ a multi-targeted mechanism, thereby reducing the risk of resistance development. This attribute is particularly valuable amid rising concerns over antimicrobial resistance (AMR). Borah et al. (2022) have shown that bimetallic nanoparticles not only enhance antibacterial potency but also reduce the dosage required for effective microbial inhibition. This dose reduction is beneficial from a toxicological perspective, especially when considering applications involving direct contact with human tissue. Another factor influencing antibacterial efficacy is the physicochemical profile of the nanoparticles. Although the particles synthesized in this study average around 308 nm, technically above the classical nanoscale (<100 nm), their narrow size distribution and stable dispersion in suspension may compensate for their larger size. According to Singh et al. (2023), surface characteristics such as charge, roughness, and functional groups play a substantial role in determining how nanoparticles interact with bacterial membranes. In that context, the monodisperse nature of the Ag-Cu NPs reported here could contribute to consistent membrane attachment and subsequent disruption.

Beyond laboratory efficacy, the real-world applicability of Ag-Cu NPs is significant. These materials can be incorporated into antimicrobial coatings for surgical tools and implants, wound dressings for infection prevention, and even antimicrobial packaging materials for food and medical supplies. Their resistance to degradation under various environmental conditions also supports their potential use in water treatment and sanitation technologies. Zhang et al. (2023) underscore the versatility of bimetallic NPs,

noting their promising role in biomedical devices due to their tunable surface chemistry and sustained antibacterial activity. Ag-Cu nanoparticles synthesized via chemical reduction offer effective antibacterial performance through synergistic ionic interactions and ROS generation. While the particles' size may exceed conventional nano-definitions, their consistent morphology and surface reactivity are sufficient to ensure biological functionality. Moving forward, detailed assessments of cytotoxicity, ion release kinetics, and efficacy against antibiotic-resistant strains will be essential in translating these nanomaterials from the lab to practical clinical and industrial applications.

4. Conclusion

The study successfully synthesized bimetallic Ag-Cu nanoparticles by chemical reduction, using sodium borohydride ($NaBH_4$) as the reducing agent and polyvinylpyrrolidone as the stabilizer. A visible color change to greenish-black confirmed nanoparticle formation. Particle Size Analyzer data revealed an average diameter of approximately 308.8 nm with a narrow, monodisperse size distribution. Antibacterial testing against *Escherichia coli* demonstrated a clear concentration-dependent effect. As nanoparticle concentration increased from 5 % to 15 %, the inhibition zone grew from an average of 7.3 mm to 7.8 mm. Although these zones remain smaller than the 27.3 mm produced by the positive control antibiotic, Ag-Cu nanoparticles show promise as an alternative antibacterial agent. Ag-Cu nanoparticles synthesized here exhibit well-controlled size and stable dispersion, together with measurable antibacterial activity. Further work on optimizing synthesis parameters, quantifying ion release, and assessing cytotoxicity will be essential steps toward their development for clinical or environmental applications.

References

A. Blanco-Flores et al. (2019). Adsorption of fluoride using bimetallic oxide nanoparticles supported on industrial waste prepared by a chemical reduction method. Desalination and Water Treatment. https://doi.org//10.5004/dwt.2019.24033

Ali A., H., Mengesha A., M., Melkamu W., W., Abebe A. (2025). Biogenic Ag-based bimetallic nanoparticle for extraordinary medicinal and photocatalytic application. *Results in Chemistry*. https://doi.org/10.1016/j.rechem.2025.102395

Duan M., Jiang L., Zeng G., et al. (2020). Bimetallic nanoparticles/metal-organic frameworks: Synthesis, applications and challenges. *Applied Materials Today*. https://doi.org/10.1016/j.apmt.2020.100564

Khan, A. Rashid, R. Younas, R. Chong. (2016). A chemical reduction approach to the synthesis of copper nanoparticles, *Int. Nano Lett*. https://doi.org/s40089-015-0163-6

Medina J., C., Garcia-Perez V., I., Zanella R. (2021) Metallic composites based on Ag, Cu, Au and Ag-Cu nanoparticles with distinctive bactericidal effect on varied species. *Materials Today Communications*. https://doi.org/10.1016/j.mtcomm.2021.102182

Saleem A., Iqbal A., Younas U., et al. (2024). Antimicrobial attributes and enhanced catalytic potential of PVA stabilized Ag-NiO2 nanocomposite for wastewater treatment. *Arabian Journal of Chemistry*. https://doi.org/10.1016/j.arabjc.2023.105545

Shumbula N., P., Ndala Z., B., et al. (2024). Dopamine Capped Silver/Copper Bimetallic Elongated Nanoparticles and Their Potential Application in Wound Healing. *Next Nanotechnology*. https://doi.org/10.1016/j.nxnano.2024.100077

Borah, P., Das, R., & Gogoi, N. (2022). Ag–Cu bimetallic nanoparticles for antimicrobial and catalytic applications: A green synthesis approach. *Journal of Hazardous Materials, 424*, 127510. https://doi.org/10.1016/j.jhazmat.2021.127510

Rai, M., Kon, K., Ingle, A., & Gade, A. (2021). Synergistic effects of silver–copper nanoparticles against bacteria and fungi: Potential as antimicrobial agents. *Applied Microbiology and Biotechnology, 105*(2), 697–707. https://doi.org/10.1007/s00253-020-11070-6

Singh, S., Kumar, P., & Singh, R. (2023). Surface properties of metal nanoparticles and their role in antibacterial activity. *Surface Interfaces, 38*, 102833. https://doi.org/10.1016/j.surfin.2023.102833

Yoon, K. Y., Byeon, J. H., Park, J. H., & Hwang, J. (2019). Antimicrobial activity of silver–copper hybrid nanoparticles against *Escherichia coli* and *Staphylococcus aureus*. *Science of The Total Environment, 688*, 1300–1306. https://doi.org/10.1016/j.scitotenv.2019.06.364

Zhang, L., Wu, H., & Yang, Y. (2023). Biomedical applications of bimetallic nanoparticles: Mechanisms and future perspectives. *ACS Nano, 17*(1), 1355–1371. https://doi.org/10.1021/acsnano.2c07318